Detection of hepatitis B virus DNA in oral fluid by using an optimised nested PCR

M.I. Micalessi[1,*], C. Gérard[1], I. Thomas[1], P. Michielsen[2], P. Van Damme[3], R. Vranckx[1], B. Brochier[1]

[1] *Scientific Institute of Public Health, Brussels, Belgium*
[2] *University Hospital of Antwerp, Antwerp, Belgium*
[3] *University of Antwerp, Antwerp, Belgium*

Background: Conventionally the detection of hepatitis B virus (HBV) DNA is carried out on serum using commercial or in house PCR assays. Oral fluid obtained by use of commercial collection devices offers several advantages compared to venepuncture: it is non invasive, painless, safe and less expensive.

Methods: Seventy-three HBV DNA positive and 74 HBV DNA negative paired serum/oral fluid samples, drawn from patients visiting university hospitals, were analysed. The detection of HBV DNA in oral fluid was carried out through an optimised nested PCR with a 95% detection limit of 457 copies/ml

Results: A clinical sensitivity and specificity of 69.9% (95%CI: 58.6%-79.2%) and 100% (95%CI: 95.1%-100.0%) respectively was achieved. A statistically significant association was found between the serum HBV DNA viral load and the detection of HBV DNA in oral fluid ($p < 0.001$). In samples with a minimum concentration of 10^5 copies/ml (high viraemia), it was possible to detect the virus in oral fluid in 91.8% (95%CI:80.8%-96.8%) of the cases. A concentration of 10^5 copies/ml being considered as the minimum level needed for the transmission of HBV via needlestick or mucosal scratch, the optimised nested PCR could be used in epidemiological surveys for mapping of active replicating carriers.

Conclusion: An optimised nested PCR for HBV detection, with a 95% detection limit of 457 copies/ml and a sensitivity of 91.8% in patients with a minimum serum concentration of 10^5 copies/ml, was developed. The use of serum remains the golden standard for individual diagnosis, monitoring of antiviral treatment or detection of occult infections. However, oral fluid sampling by oracol collection device offers through all its advantages versus venepuncture a good alternative in epidemiological and surveillance surveys.

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Screening for Influenza A H5N1 Haemagglutinin and Neuramidase Mutations Using the New Alignment & Mutation Analysis Tool in Bionumerics

B. Pot*, J. Goris, M. Vauterin, K. Janssens, P. Vauterin, L. Vauterin

Applied Maths NV, Sint-Martens-Latem, Belgium

The haemagglutinin (HA) and neuramidase (NA) genes are considered to be responsible for the major part of the antigenic variability of influenza A viruses. Reportedly, specific amino acid changes might even serve as molecular markers for the pandemic potential of avian influenza isolates. Here, we illustrate the use of this versatile and powerful tool with publicly available sequences and trace files. The latter were automatically assembled into contigs and imported with the batch assembly plugin. Optimized settings for trimming and assembly minimized manual editing. Strain information was parsed along with the import and all data were stored in a single relational database.

Using this plugin, we were able to assemble 22,000 influenza A genome sequence trace files from the NCBI Trace Archive (http://0-ww.ncbi.nlm.nih.gov.catalog.llu.edu/Traces/trace.cgi) in less than 30 minutes. For the HA and NA gene sequences of influenza A virus serotype H5N1, a multiple alignment was created and the nucleic acid sequences were translated into amino acids. Mutations were searched relative to a consensus sequence, which made ''hot spots'' for mutations to become immediately apparent. For each mutation, its position, type (silent, missense, indel), nucleotide change and amino acid change was listed. When browsing through the mutation list, the cursor jumps automatically to the corresponding position on the alignment and curves, allowing the researcher to visually evaluate the mutation in its context.

Overall, performing the complete mutation analysis within a single software package offers much added flexibility, in comparison with online analysis tools.

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Universal Virus Detection

C. Uhlenhaut*, A.M. Sierra-Honigmann, S. Nanda, S. Tang, P.R. Krause

FDA/CBER, Bethesda, MD, USA

Reliable and fast assays are prerequisite for rapidly identifying infectious agents such as emerging viruses, zoonotically transmitted or otherwise rare and unusual viruses in order to take appropriate epidemiologic actions and/or to choose the best treatment. However, detection and identification of viruses without specific knowledge about their genome can be challenging.

We developed an assay that combines virus capsid preparation with generic PCR. The first step is the physical and biochemical purification of a given sample by targeted digestion of contaminating host nucleic acids. This is followed by a degenerate oligonucleotide primer (DOP) PCR. The primer population is optimized for the detection of virus-sized genomes. Products can be identified by cloning and sequencing or by subjecting the PCR products to high throughput sequencing. Various DNA viruses (including HSV, VZV, SV40, AAV, EBV, parvoviruses and hepatitis B) and RNA viruses (including HTLV-1 and HTLV-2, several animal retroviruses, poliovirus, hepatitis A, a human corona virus, human metapneumovirus and influenza virus) were detected in cell cultures and clinical samples.

This novel technique requires no prior sequence information to amplify viral genomes, but nonetheless be able to amplify at least a portion of each viral genome. It can detect viruses in a variety of samples using only one aliquot